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Survey of the Cymothoid Parasites Associated with some Estuarine Fishes (Actinopterygii) from the Upper Reaches of the Bonny Estuary Nigeria: Their use in Ecotoxicological Studies

Ugbomeh AP[1]*, Nwosu OR[2]

[1]Animal and Environmental Biology Department, Rivers State University of Science and Technology Nkpolu, PMB 5080, Port Harcourt, Nigeria

[2]Institute of Geosciences and Space Technology, Rivers State University of Science and Technology Nkpolu, PMB 5080, Port Harcourt, Nigeria

Research Article

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***For Correspondence**

Ugbomeh AP, Animal and Environmental Biology Department, Rivers State University of Science and Technology Nkpolu, PMB 5080, Port Harcourt, Tel: +2348033104090

E-mail: Ugbomeh.adaobi@ust.edu.ng

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ABSTRACT

Five hundred and forty two (542) fishes belonging to 9 species (*Pomadasys peroteti*, Tilapia spp, *Arius gigas*, *Elops lacerta*, *Eucinostomus (Gerres) melanopterus*, *Pseudotolithus elongatus*, *Liza grandisquamis* and *L. falcipinnis*) were collected from 5 landing sites along the upper reaches of the Bonny river in Nigeria and examined for macro-ectoparasites. Parasites isolated were *Cymothoa exigua*, *C. hermani*, *Nerocila monodi*, an unidentified *Nerocila* spp and *Lironeca vulgaris*. Total prevalence for all parasites was 37%. For each parasite, the host fish, parasitic specificity and prevalence are given. *P. peroteti* and Tilapia spp had the highest parasite prevalence. The Cymothoa species were found in the mouth replacing the tongue, in the pharynx attached to the gill arch and externally on the skin of *P. peroteti* and Tilapia, *Lironeca vulgaris* was found in the mouth while the Nerocila species were found on the skin. A toxicological survey of the concentration of heavy metals (Cd and Pb) in fish and parasite showed the absence of heavy metals in all parasites while the fish had concentrations above recommended permissible limits. There was no significant difference in the prevalence among stations.

INTRODUCTION

Every living thing has its own group of parasites so there has been an increasing demand for knowledge about the nature, biology and control of fish diseases [1]. There is also the use of aquatic fauna in bio monitoring and the economic importance of fishing as a source of employment and sports that information on the knowledge of fish parasitology has become very important [2,3]. Parasites affect the tissue of infected fishes, their economic value, and their environment.

Fishes have been used as bio monitors of pollution and contaminations of the environment. However, there are different opinions by some environmental parasitologists that parasites (especially the macro parasites) could be better indicators of pollution making parasites of fin fish a potential valuable indicator of ecological impacts [4]. The chronic exposure of organisms to pollutants causes biochemical, physiological and behavioural host changes that ultimately can influence the prevalence and intensity of parasitism [5]. As a result, they infered that pollutants might promote increased parasitism in aquatic animals, especially fish, by impairing the hosts immune response or favouring the survival and reproduction of intermediate hosts or depauperate the parasite population. Sures [6] in some experimental studies observed that the number of ectoparasites such as trichodinid ciliates and monogeneans increased significantly in the gills following exposure to pollutants. Therefore, the incidence or prevalence of a particular parasite on a given fish in a known area could indicate environmental contamination. The thorny or spiny headed

worm (acanthocephalans) parasitizing fish, have been shown to accumulate heavy metals at concentrations that are many times higher than those recorded in the host tissue [7]. The use of fish parasite as bio-indicators is on the increase, either because of their physiological response to aquatic contaminants, and or because of their ability to accumulate particular toxic agents [5]. The cymothoid parasites are large, easy to observe and collect and occur in both marine and freshwater shallow water fishes [8].

Fish farming or aquaculture and fishing has remained a major economic activity of many communities in riverine regions of Nigeria with private individuals as well as industries setting up fish farms without a corresponding increase on the knowledge and biology of their parasites both in relation to the environment and man, thus making this study very useful. Sarig [9] stated that this lack of information and knowledge as the reason infections of fishes by parasites often escape unnoticed and even when noticed are not properly identified and documented. Recent researchers in the Niger delta of Nigeria such as [10-19] have made tremendous advances in the study of fish parasites, mainly in study of helminths and brief mentions of isopods as a group. However, there remains a large vacuum in the knowledge of fish diseases and pathology in Nigerian waters. Epidemiology of fish diseases is therefore necessary to provide effective control in wild and cultured fish. Due to incessant crude oil spills and blow outs in the Niger Delta areas, and concomitant dredging activities, many scholars believe that fisheries and biodiversity of aquatic animals are adversely affected [20]. Thus, a study of fish and their parasites in the area will provide data on the fish species adversely affected, degrees of infection and interaction of the organisms within polluted and non- polluted waters. The idea is to promote the possible use of fish parasites as indicators of water pollution. Due to poor knowledge of fish parasites, fish industries suffer huge financial calamities each year to parasites and diseases of fishes, which are largely responsible for the deaths of fish and hatcheries [21-23]. Of the four basic culture systems in Nigeria, none has escaped the menace of fish parasites [24]. A yet unreported incidence of fish parasite on a farm in Agbor, Delta State of Nigeria showed very low productivity of Tilapia infected with cestodes. Therefore on a daily basis, the problems of parasites both in the cultured and wild species of fishes are encountered. Knowledge of fish parasites in Rivers State is necessary for ecological studies, economic health of our people, in aqua studies, and as database for future Environmental Management studies. Cymothoid parasites are isopods of family Cymothoidae. They are macroparasites that can grow up to 7 cm. Brusca [25] describes them as protandric hermaphrodites with mouth part modified for parasitic lifestyle, known to parasitize wild marine, brackish and freshwater fish species of commercial importance in shallow waters. This study conducted over a 5 month period from July to November 2015 hopes to establish the local species and to promote the possible use of cymothoid parasites as indicators of heavy metal pollution.

MATERIALS AND METHODS

Description of study area

The collection sites are in Rivers State of Nigeria in the Niger Delta region designated as Afikpo watersides (N 04° 64′ 38.9″ and E 006° 59′ 26.5″), Abonnema watersides (N 04° 46′ 30.4″and E 007° 00′ 15.5″) and Creek road water sides (N 04° 48′ 33.2″ and E 007° 01′ 25.2″) in Port Harcourt Local Government Area, and Ogbogoro watersides (N 04° 50′ 15.4'' E 006° 55′ 31.8″) and Iwofe water sides (N 04° 48′ 33.2″ E 006° 55′ 42.0″) in Obio/Akpor Local Government Area in Rivers State. Afikpo and Creek road water sides are heavily polluted with sewage and refuse which are dumped into the river. Iwofe water side has high human settlement and Eagle cement factory close to it, and as such, receives organic and industrial waste from its immediate surroundings. Abonnema and Creek road watersides have high degree of water traffic disturbances and high human settlement. Ogbogoro waterside is relatively calm, with occasional vehicular movement. These three sites (Ogbogoro, Iwofe and Abonnema watersides) are on the shore of the Bonny river while two sites (Afikpo and Creek road watersides) are situated on creeklets of the Bonny river **(Figure 1).**

Collection of samples

Six families of fish were collected during the sampling period: Pomadacyidae (*Pomadasys peroteti*), Cichlidae (Tilapia species) Gerreidae (*Eucinostomus (Gerres) melanopterus*), Scianidae (*Pseudotholitus elongatus* and *Pseudotholitus senegalensis*), Mugilidae (*Liza falcipinnis* and *Liza grandisquamis*), Ariidae (*Arius gigas*) and Elopidae (*Elops lacerta*) of which no parasites were found in the mugilidae and ariidae. They were purchased from fishermen using Cast nets and beach seines operating around the landing sites. The sampled fishes were collected and transported in polythene bags to the University. The fishes were not separated into males and females. They were ice frozen and examined the same day in the laboratory. Fish samples from the study sites were collected monthly from July to November, 2015. The sampled fishes were separated in to groups and identified using identification sheets by [26] using morphometric and meristics characters. The identified fishes were measured to the nearest mm and checked for macro ectoparasites starting from the skin/body surface, gills, mouth, operculum, anus and the fins. The isolated parasites were identified using guides provided by [25-29]. Basically the shape of cephalon, its position in the 1st pereonite, the shape of the pleonite, the pleotelson and uropod were all useful in identification using available keys. The extracted parasites were preserved in specimen bottles containing 70% ethanol.

Five samples of *Pomadasys peroteti* from Ogbogoro station of size range 10 to 14.7 cm and corresponding five samples of *Cymothoa exigua* of size 1.6 to 2.0 cm were used for heavy metal analysis as it appeared to have a greater affinity for infection as more than one parasite was found in them. Samples were prepared according to methods by [30] and analyzed by flame atomic absorption spectrophotometer (AAS model GBC Avanta Pm AAS).

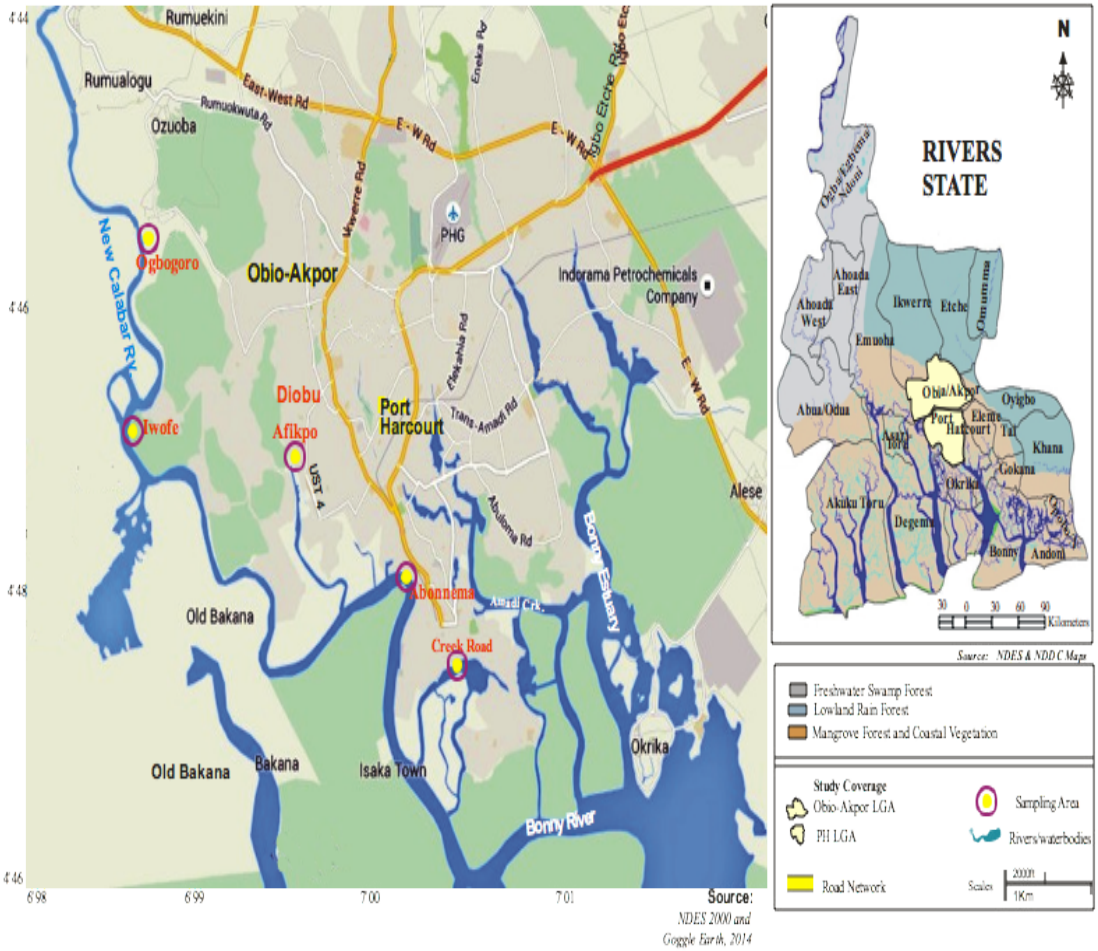

Figure 1. Map showing the Study Areas- Creek road, Abonnema, Afikpo, Iwofe and Ogbogoro water sides in Rivers State.

Means, standard deviations and ANOVA (Analysis of variance) and Post –hoc testing of means by Tukey was done using Microsoft excel and SPSS 13.

RESULTS

The fish species were identified as; Pomadasyidae (*Pomadasys peroteti*), Cichlidae (Tilapia spp), Gerreidae [*Eucinostomus (Gerres) melanopterus*], Scianidae (*Pseudotholitus elongatus* and *Pseudotholitus senegalensis*), Mugilidae (*Liza falcipinnis* and *Liza grandizquamis*), Ariidae (*Arius gigas*) and Elopidae (*Elops lacerta*). The *ectoparasites* were found in *Pomadasys peroteli*, *Tilapia* spp, *Eucinostomus (Gerres) melanopterus*, *Pseudotholitus elongatus* and *Elops lacerta*. No parasite was isolated from the Mugilidae and the Ariidae.

The ectoparasite isolated were all Isopods of family Cymothoidae. A total of five hundred and forty two (542) fishes were examined, one hundred and ninety-eight (198) of the fishes were infected with a prevalence of 37%. Total number of parasite from infected fishes was Two Hundred and Forty-Eight (248) because some had male and female in one fish. The cymothoid parasites isolated, belong to three genera and five species namely; *Cymothoa hermani*, *Cymothoa exigua*, *Lironeca vulgaris*, *Nerocila monodi* and Nerocila spp (*unidentified*). The parasites were found on the skin, mouth and pharynx(on the gill arch) but mostly in the mouth and pharynx.

N. monodi was found on the body of Tilapia spp as shown in **(Plate 1).** The colour is a deep tan with 3 distinct longitudinal dark bands. They were found on the skin (lateral side) of the host Tilapia. The other Nerocila spp had a different coloration from *N. monodi* with large prominent eyes **Plate 1 (b).** They were found on skin (under belly) of *Tilapia*. There were no distinct longitudinal dark bands on the dorsal surface.

Plate 1 a) *Nerocila monodi*. (Arror bar 0.8 mm)

Plate 1 b) Nerocila spp on *P. peroteli*

Cymothoa hermani **(Plate 2)** was found in the mouth of Tilapia and *Pomadasys* where they had eaten out the tongue taking its place as well as the pharynx. Found singly or in pairs (1 male or male and female) in the pharynx of *Pomadasys*, mouth of Tilapia and skin of *Elops* and *Gerres*. When single, they were found in the mouth or skin. In pairs the second one was found on the gill arch or in the pharyngeal region.

Plate 2. *Cymothoa hermani* from the mouth of *Pomadasys peroteti. (*Bar 1.2 cm).

Cymothoa exigua **(Plate 3)** was found on the skin of *Pomadasys*, Tilapia, *Elops* and *Gerres*, as well as in the pharynx and mouth

Plate 3. *Cymothoa exigua* on the upper lip of Tilapia. (Bar 0.9 mm).

Prevalence of parasites

The percentage prevalence of parasites in the family Gerreidae was 65%, Pomadacyidae 65%, Cichlidae 57% and Sciaenidae 5%. A total of 61 and 38 fishes were examined in the families Muglidae and Ariidae respectively, both had zero prevalence **(Table 1).** The overall parasite prevalence of examined fishes was 37%. The parasite isolated were *Nerocila monodi*, *Cymothoa hermani*, *Cymothoa exigua* and *Lironeca vulgaris* **(Plate 4)** from *E. melanopterus*, *P. peroteti*, Tilapia spp, *P. elongatus* and *E. lacerta*.

Table 1. Parasitological index of the cymothoid parasites isolated for the period of study.

Host/Parasite Species	Number of Fish Examined (NFE)	Range of fish size (cm) (LS)	Number of fish infected (NFI)	Prevalence (%) NFI/ NFE x 100 (P)	Location in/on host (LO)	Place of Collection (PC)
Family: Gerreidae	54	4-11	35	65	Skin (Under belly, Lateral sides and around the fish lips)	Creek Road, Abonnema, Afikpo, Ogbogoro
E. melanopeterus						
Cymothoa exigua						
Cymothoa hermani						
Family: Mugilidae	61	7 – 15	0	0	-	Abonnema, Afikpo, Creek Road, Ogbogoro
Liza falcipinnis						
Liza grandisquanis						
Family: Pomadasyidae: *Pomadasys peroteti*	119	6-14	77	65	Mouth, Pharyngeal cavity, Skin	Creek Road, Iwofe, Abonnema, Afikpo, Ogbogoro
Cymothoa hermani						
Lironeca vulgaris						
Cymothoa exigua						
Family: Cichlidae	173	7-12	98	57	Mouth, Pharyngeal cavity, Side of mouth, Skin	Iwofe
Tilapia spp						
Cymothoa exigua						
Cymothoa hermani						
Nerocila monody						

Family: Scianidae *Pseudotolithus elongatus* *Cymothoa exigua*	62	7-13	3	5	Mouth	Creek Road, Afikpo, Abonnema
Family: Ariidae *Arius gigas*	38	7-15	0	0	-	Creek Road, Abonnema, Iwofe
Family: Elopidae *Elops lacerta* *Cymothoa exigua*	35	7-14	19	54	Skin, mouth	Ogbogoro
Total	542		198	37		

Plate 4. *Lironeca vulgaris* (Bar 1 cm) *L. vulgaris* was found in the pharynx of *Pomadasys.*

The percentage infection varied among the collection sites **(Figure 2).** These variations were found to be significantly different for *P. peroteti* and Tilapia but not for in *E. melanopterus* **(Table 2)** at p = 0.05 (TUKEY). Infection in Tilapia grouped the stations into three; Afikpo and Creek road water sides in the creeklets, Abonnema and Iwofe, and Ogbogoro watersides. The highest incidence of infection for *P. peroteti* was at Abonnema and Iwofe, for Tilapia spp was Afikpo and Creek road and at Ogbogoro for *E. melanopterus* **(Figure 2).**

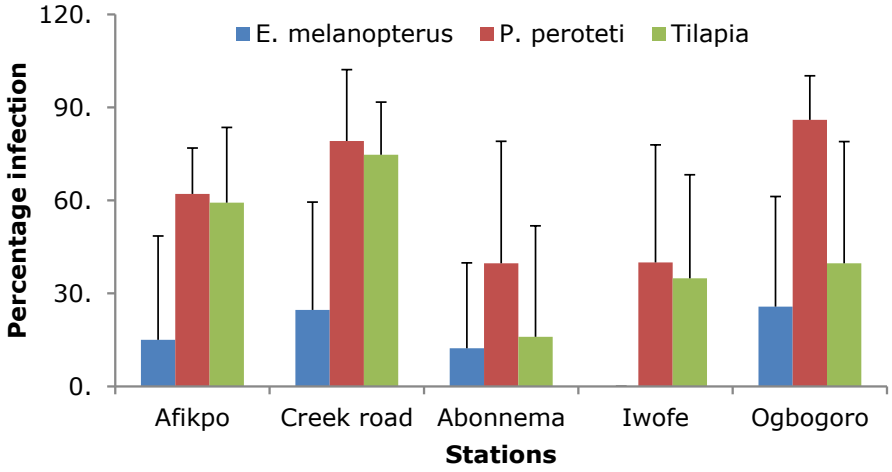

Figure 2. Spatial variation in the Mean and standard deviation of percentage infection in *E. melanopterus,* Pomadasys and Tilapia.

Table 2. Means ± SD of percentage infection with cymothoid parasites at the five landing sites (Tukey mean separation).

Locations	Fishes/percent infection		
	E. melanopterus	***P. peroteti***	**Tilapia**
Afikpo	15.00 ± 33.54[a]	62.10 ± 14.79[a]	59.27 ± 24.27[b]
Creek road	24.67± 34.77[a]	79.14 ± 23.07[a]	74.75 ± 16.98[b]
Abonnema	12.31 ± 27.52[a]	39.72 ± 39.33[a]	16.00 ± 35.78[a]
Iwofe	0.00 ± 0.00 [a]	40.00 ± 37.91[a]	34.86 ± 33.41[a]
Ogbogoro	25.71 ± 35.57[a]	86.00 ± 14.22 [b]	39.70 ± 39.29 [ab]

Note: Along column, same superfix shows means that are not significantly different.

Heavy metal analysis done on muscle of *Pomadasys peroteti* were 1.06 ± 0.58 (range of 0.47 - 2.74 mg/kg) for Pb and 0.30 ± 0.20 mg/kg (range of 0.33 - 1.09 mg/kg) for Cd. The concentration in the whole specimens of *Cymothoa exigua* retrieved from the mouth were below detection limit of 0.001 mg/kg in all the *C. exigua* examined **(Table 3).**

Table 3. The concentration of heavy metal in *P. peroteti* and *C. exigua* retrieved from their mouth.

Concentration mg/kg	P. peroteti	C.exigua
Pb	1.06 ± 0.58	Below detection limit
Cd	0.30 ± 0.20	Below detection limit

DISCUSSION

Fish species and parasites

The main food fish available for sampling were *Pomadacyidae* (*Pomadasys peroteti*), Cichlidae (Tilapia species) Gerreidae (*Eucinostomus (Gerres) melanopterus*), Scianidae (*Pseudotholitus elongatus* and *Pseudotholitus senegalensis*), Mugilidae (*Liza falcipinnis* and *Liza grandisquamis*), Ariidae (*Arius gigas*) and Elopidae (*Elops lacerta*) (of which no parasites were found in the mugilidae and ariidae) as the market women bought whatever was caught. They are the common estuarine fish usually marked fresh. The market women were observed picking out the parasites (which they called "insect") from the skin and mouth of the fish before displaying them for sale. This is because they found the parasites offensive as it did not look nice on the fish.

The total percentage prevalence was 37%. Bunkley and Williams [31] working on isopods associated with Venezuelan marine fishes, and [32] that worked on the Cymothiodal (Crustacean, isopoda), parasites on marine fishes from Algerian fauna recorded approximately 4 - 29% prevalence. Inyang [18] also recorded isopods in a tilapia *S. melanotheron*, in a goby *Bostrychus africanus* and in Sphyraena spp. In this report however cymothoids were found in pomadasyids, tilapia, sciaenids, gerreids, and elopidae. A comprehensive study of the parasites in the Bonny Estuary is advised to include all available fish to complement this as baseline data.

The zero prevalence observed in the Mugilidae and Aridae could be as a result of host specificity. Probably, there is some kind of a chemical substance in the body of these fishes that repels the parasite or do not allow them to infect these fishes. Host specificity has been observed by [23] while working on the parasite infections and diseases of fish in Africa. Sarig [9] noted host specificity while working on the diseases of fishes and the prevention and treatment of warm water fishes.

It should also be noted that amongst the Cymothoid parasites encountered in this study had a distribution or location in/on the fish host that varied. Nerocila species were found on the skin of the fish host – cichlids. *Cymothoa hermarni* and *Cymothoa exigua* were found on the skin of the Gerreidae, Pomadacyidae, Cichlidae and Sciaenidae and in the mouth and pharynx of pomadasyidae, cichlids and elopidae. *Lironeca vulgaris* was found in the mouth of Pomadacyidae. The Pomadasyids appear to have a greater affinity for infection as more than one type of parasite was found on them. More work need to be done to understand site specificity among these parasites. The position of the parasites on fish makes for easy collection without killing or destroying the fish. It is suggested therefore that to isolate cymothoids from estuarine food fish in Nigeria for studies, *E. melanopterus*, Tilapia, *E. lacerta* are important hosts in decreasing order. The parasites were also observed singly and in pairs in some. Cymothoids are known protandric hermaphrodites. When single they occurred in the mouth and were male. As a pair, the new male (smaller in size) remains in the pharynx and the first which is larger becomes the female. The presence of a second male has been documented by [25] to trigger the change of the first male into a female during their next molt.

Heavy metal analysis

Khan and Thulin [5] noted that the use of fish parasites as bio indicators is on the increase either because of their physiological response to aquatic contaminants or because of their ability to accumulate particular toxic agents. Acanthocephalans parasitizing fin fishes have been shown to accumulate heavy metals at concentrations that were many times higher than those recorded in the host tissue [7]. Bearing this in mind, heavy metal analysis was done on the parasite *Cymothoa exigua* and their fish host. Although the heavy metals like Pb and Cd were detected in the fish hosts, they were not detected in the cymothoid parasites collected from the mouth. Acanthocephalans are gut parasites of the fish host, so they are almost feeding on the food from same environment like their fish hosts as they derive their nutrients mainly from the chyme and raw food of the fish [7]. As such, heavy metal accumulation could easily reflect on the body of the parasite. The cymothoids in the mouth mainly depend on the mucus and assimilated food of the fish as such only a negligible portion of the raw food gets to the isopods. Brusca [25] has argued that the cymothoids in the mouth appear to be non-feeding (apart from eating up the tongue). The parasite in the gills however were pink in colour showing they had fed on some blood material. It is however suggested that heavy metal analysis will be done on those that are in the gills and further research is advised. Expected results should have reflected the heavy metal load of the fish in the parasite. Also, the cymothoids are arthropods with chitinous exoskeleton and it is possible that this acted as a barrier to assimilation of heavy metals from the environment even as ectoparasites or they had preferential ability to regulate Cd and Pb. It appears that as the parasite was collected from the mouth, it may not have been feeding on blood as the parasites on the gills which were pink indicating some form of blood meal. Further investigation is needed to establish presence or absence of heavy metals in parasites obtained from the gills as well as on the use of gut parasites as bio monitors.

In conclusion, this five month survey has shown that estuarine fishes from five landing sites in Port Harcourt (*P. peroteti*, *E. lacerta*, *E. melanopterus*, and Tilapia) are host to isopod parasites of family *cymothoidae - Cymothoa exigua*, *C. hermani*, *Nerocila monodi*, *Nerocila spp* and *Lironeca vulgaris*. *P. peroteti* and Tilapia had the highest infestation rates in the mouth and pharynx. Toxicological studies show that the parasites from the mouth may not be useful as bioindicators of Pb and Cd. Further investigation is recommended for all heavy metals in parasites collected from the pharynx feeding on the gill arch.

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